Butamax™
Advanced
Biofuels LLC

A joint venture between BP and DuPont

10 December 2012

Mr. Dave Kortum
Office of Transportation & Air Quality
US Environmental Protection Agency
via e-mail

Dear Mr. Kortum

Referring to Butamax's 09 June 2011 application to register isobutanol as a gasoline additive, attached please find supplemental Exhibit I presenting a summary of the available pharmacokinetic / toxicokinetic studies on isobutanol. This exhibit is presented in response to EPA's requirement for pharmacokinetic (PK) studies on 'neat' oxygenates used as oxyfuel components per Alternative Tier 2 Health Effects testing provisions of the 211(b) Fuel and Fuel Additives regulations.

These studies provide basic data on absorption and elimination of isobutanol and identify the principal metabolites. The studies consistently demonstrate that isobutanol is rapidly absorbed by oral and inhalation routes and is metabolized to isobutyraldehyde and isobutyric acid in both laboratory animals and humans. We believe these studies continue to indicate that isobutanol and its proposed fuel blends present minimal toxicity and would not pose a potential hazard to human health.

We appreciate the opportunity to make this supplemental submission and look forward to continuing work with the Agency on Butamax's isobutanol registration application. We request the opportunity to meet at your earliest convenience to further discuss EPA's review of our application.

Sincerely,

[redacted]

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CAA 211(b) Alternative Tier 2 Literature Search and Summary of Isobutanol Pharmacokinetics Studies

Supplemental Exhibit I
for Butamax's 09 June 2011 Application
to Register Isobutanol as a Gasoline Additive

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CAA 211(b) Tier 1 Literature Search and Summary Information for 24 Compounds Unique to 16% Isobutanol-blended Fuel Emissions

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Toxicokinetics, Metabolism and Distribution of Isobutanol

Isobutanol has been demonstrated to be metabolized to isobutyraldehyde and isobutyric acid in humans, rats, and rabbits, through the alcohol and aldehyde dehydrogenase enzyme system. Much of the available data has been summarized in the OECD SIAR, 2004 from which robust summaries are attached (Appendix A).

<u>Human studies</u>

Rudell et al, 1983 measured levels of isobutanol, isobutyraldehyde and isobutyric acid in blood and urine from subjects who ingested isobutanol in an ethanol/water vehicle over two hours. At the end of the consumption period blood concentrations of isobutanol, isobutyraldeyde and isobutyric acid were, 4, 4, and 17µmol/L whole blood respectively, demonstrating that isobutyric acid was the major metabolite. The 4µmol/L of isobutanol correlated with an oral dose of approximately 5mg/kg reported in previous studies. The presence of ethanol in the test beverage altered the rate of isobutanol metabolism due to competition for metabolic enzymes but did not affect the metabolic process. By 2 hour post-dose, isobutanol had decreased in the blood to 1µmol/L and no isobutanol was present at 9 hours. Isobutyraldehyde levels increased slightly by 2 hours post-dose and remained stable for 9 hours and isobutyric acid gradually decreased to 10µm/L at 9 hours post-dose. Urine concentration time curves were constructed for isobutanol, isobutyraldehyde, isobutyric acid and its metabolites (proprionaldehyde, proprionic acid and succinic acid). Urinary concentrations of isobutanol peaked at 1 hour post-dose, isobutyraldehyde at 8 hours, and isobutyric acid at the end of the 2 hour treatment period. Urinary levels of proprionaldehyde followed those of isobutyraldehyde; levels of proprionic acid rose after the treatment period ended and plateaued between 2 and 8 hours and succinic acid followed the proprionic acid elimination curve.

Bilzer et al., 1990, monitored blood concentration levels of isobutanol from 6 subjects (2 males, 4 females) who ingested an 1875mg/L isobutanol/30% ethanol beverage constituting an approximate oral dose of 5 mg/kg isobutanol and 0.80g/kg ethanol over 30 minutes. Blood concentration of isobutanol peaked 45 minutes after start of treatment and decreased over 240 minutes. The half-life of isobutanol in the presence of ethanol was 1.46 hours. Peak serum levels of isobutanol were reported as approximately “6 mg/kg” [sic] and blood ethanol levels were approximately 1%. No excreta were collected.

<u>Human *in vitro* studies</u>

A study by Ehrig et al., 1988, examined the role of human alcohol dehydrogenase (ADH) isozymes in the metabolism of isobutanol. Human liver ADH isozymes Class I, II, III were prepared from tissue of 2 healthy, suddenly deceased donors. A single dose of 10µM isobutanol was administered with study duration of 10 minutes. Class I ADH kinetic rate constant (Km) was 33µM and a Vmax of 0.19 IU/mg protein for isobutanol resulting in activity of 0.14 IU/mg. Class II ADH activity was much lower at 0.0004 IU/mg and no Class III activity was detected. Thus, Class I ADH isozyme is primarily responsible for oxidation of isobutanol in the human liver.

Sinclair et al, 1990 used human hepatocytes (4 donors) as well as supernatants of rat liver and chick embryo to compare relative metabolic rate constants for ADH activity. Human liver supernatant activity has Km = 0.04 - 0.11µM and Vmax = 0.68 – 0.86 µmol min^{-1} g wet wt^{-1} Rat liver values were Km = 0.05µM and Vmax = 1.07 µmol min^{-1} g wet wt^{-1} and chick embryo values were Km = 0.22µM and Vmax = 0.29 µmol min^{-1} g wet $wt^{-1.}$

Wilkens and Stewart, 1987 measured metabolism of isobutanol (single exposure, 1nM) by ADH in homogenate of human skin samples. ADH activity was 103.7nM/mg protein-minute.

<u>Animal studies</u>

Isobutanol metabolism was investigated in male and female Wistar rats by intraperitoneal injection, liver perfusion and *in vitro* rat liver supernatant (Hedlund and Kiessling, 1969) with test mixtures of isobutanol/ethanol. The ratio between concentration of ethanol and isobutanol was 100:1 in liver homogenate experiments and approximately 10:1 in the *in vivo* experiment. Intraperitoneal injection resulted in isobutanol levels in blood of 0.1mg/ml at 15 minutes post-dose with only a slight decrease over 7 hours. Blood ethanol levels peaked at 0.7mg/ml and decreased over 5 hours to baseline. Blood levels of isobutanol did not start to decline appreciably until ethanol levels decreased to 0.2mg/ml. Thus metabolism of isobutanol in vivo appeared to be retarded by simultaneous oxidation of ethanol. However, rat liver perfusion results indicated isobutanol was metabolized at a rate of 0.06mM/gram liver during the first 30 minutes, more rapidly than ethanol and in vitro at a rate of 0.2mM/g rat liver homogenate in 30 minutes, also more rapidly than ethanol.

Saito, 1975 administered a single 2ml/kg dose of isobutanol alone or 2ml/kg isobutanol followed by water containing 20% (v/v) isobutanol to anaesthetized rabbits. Arterial blood levels of isobutanol in one group and excretion of isobutanol and metabolites in urine and expired air from a separate group of rabbits were monitored. Isobutanol blood levels peaked at 1 hour post-dose to approx. 0.8mg/ml and decreased to near zero at 4 hours post-dose. Very little isobutanol (0.5%) was excreted in urine or exhaled air. Urinary levels of isobutyraldehyde = 0.12mg/ml and only trace amounts of isobutyric acid were present. An unexplained level (1.6 mg/ml) of a substance co-eluting with isovaleric acid was found in the urine.

The only inhalation study available for isobutanol is a respiratory bioavailability study performed by Poet, 2003. A rat with an indwelling jugular catheter and fitted in a whole-body plethysmograph to measure ventilatory movements was placed in a gas-uptake chamber. The chamber was charged with 2000ppm isobutanol and maintained for 2 hours. Blood concentrations of isobutanol and isobutyric acid were measured over 90 minutes. Isobutanol levels in whole blood increased up to 278µM at 15 minutes and declined over the remaining time to 155µM at 90 minutes. Isobutyric acid levels increased up to 93µM at 25 minutes and declined to 40µM by 60 minutes.

Modeling of isobutanol (2-methyl-1-propanol) distribution between blood and tissues

Beginning with published data on partition coefficients for a large number of volatile organic compounds (VOC) Abraham and colleagues (2005; 2006a, b) have developed equations to predict the distribution of VOC in air to blood and tissues using a linear free energy analysis method. The K value is derived from concentration of compound in blood/concentration of compound in air. The authors demonstrated that the log K values for air - blood partition and air - brain partition in humans and rats are sufficiently similar to be averaged for calculation purposes. Reported log K values for isobutanol air to blood partition are 2.89 (human) and 2.94 (rat); average 2.92 and for air – brain 2.61 (human) and 2.94 (rat), average 2.78. Using the averaged values, the calculated log $P_{\text{blood-brain}}$ is - 0.14 for distribution between blood and brain (Abraham et al., 2005, 2006a). Similarly, distribution coefficients (log P_{muscle}) from blood to muscle can be calculated by combining log K_{muscle} values with values for air and blood. Log K values for air-muscle = 2.54 (human) and 2.93 (rat), average 2.74. Using average values of log $K_{\text{air-muscle}}$ 2.74 and log K_{blood} 2.92, the log $P_{\text{blood-muscle}}$ can be calculated as – 0.18 for isobutanol (Abraham et al, 2006b). Developing these modeling methods to analyze distribution of compounds between blood and tissues is valuable for understanding the potential toxic effects of VOC.

These studies demonstrate that isobutanol is rapidly absorbed by oral and inhalation routes of exposure and is metabolized to isobutyraldehyde and isobutyric acid in animals and humans. Isobutanol has been demonstrated to induce minimal toxicity *in vivo*.

References

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Ibid, (2006a) Air to brain, blood to brain and plasma to brain distribution of volatile organic compounds: Linear free energy analyses. European J Medicinal Chemistry 41 (4): 494-502

Ibid, (2006b) Air to muscle and blood/plasma to muscle distribution of volatile organic compounds and drugs: Linear free energy analyses. Chemical Research in Toxicology .19(6): 801-808

Appendix A: Isobutanol: Toxicodynamic/toxicokinetic Robust Summaries from OECD SIDS dossier 2004

5.0 Toxicity

5.10 Toxicokinetics Section B pp 80-87

B. Toxicodynamics, toxicokinetics

(a) Preferred value

Species:	human
Strain:	N/A
Sex:	Not available
Route of Admin:	oral
Exposure Period	Two hours
Freq. of Treatment:	Single
Duration of Test	Eleven hours
Exposure Concentration	Not reported. Administered as Isobutanol and Ethanol in water to produce a blood isobutanol level of 4 µmol/L whole blood at end of dosing period.
Control Group:	None (biological samples taken prior to exposure)
Method:	In an effort to understand the elimination kinetics of aliphatic alcohols found in alcoholic beverages, research was conducted with human subjects. Test subjects consumed isobutanol in a ethanol/water vehicle over a two hour time period. Blood and urine samples were collected prior to consumption, at the end of the two-hour consumption period, at one, two, eight (urine only), and nine hours after the end of the exposure period. Similar experiments resulted in an oral dose of approximately 5 mg/kg isobutanol. The blood and urine samples were mixed, treated with β–glucuronidase, deproteinated, and esterified with methanol or ethanol to detect the acid or aldehyde "down-stream" metabolites. Blood concentration-time curves were constructed for isobutanol, isobutyraldehyde, and isobutyric acid. Urine concentration-time curves were constructed for isobutanol, isobutyraldehyde, isobutyric acid, propionaldehyde, propionic acid, and succinic acid. The last three metabolites

(propionaldehyde, propionic acid, and succinic acid) are the known metabolites of isobutyric acid.

Year: 1983
GLP: no
Test substances: isobutanol, ethanol
Purity: not provided
Result: The blood concentrations of isobutanol, isobutyraldehyde, and isobutyric acid were approximately 4, 4, and 17 µmol/L at the end of the consumption period, clearly demonstrating that isobutyric acid was the major metabolite of isobutanol metabolism. While the addition of ethanol to the test beverage definitely altered the rate of isobutanol metabolism (via a competition for metabolic enzymes), the presence of ethanol did not affect how isobutanol was metabolized. Blood levels of isobutanol decreased over the next two hours while the isobutyraldehyde levels slowly increased in the blood. Isobutyric acid levels also decreased after the end of the consumption period. Urinary concentrations of isobutanol peaked at the one-hour post-exposure time point. Urinary levels of isobutyraldehyde peaked at the eight-hour post-exposure time point. Urinary levels of isobutyric acid peaked at the end of the two-hour exposure period. Urinary levels of propionaldehyde roughly followed those for isobutyraldehyde with peak levels of approximately 8 µmol/L. Urinary levels of propionic acid rose after the exposure period ended with plateau levels between 2 and 8 hours of approximately 60 µmol/L. Urinary levels of succinic acid roughly followed the propionic acid urinary elimination curve with peak levels of approximately 30 µmol/L. A diagram was provided in the paper describing the further metabolism of isobutyric acid, ending with propionic acid. The formation of succinic acid from propionic acid is proposed based on the known intermediate metabolism of propionic acid via the citric acid cycle.

Isobutanol, isobutyraldehyde, and isobutyric acid blood levels found following isobutanol administration.

Sampling Time (hours)	Isobutanol*	Isobutyraldehyde*	Isobutyric Acid*
Beginning of dosing	0	0	0
End of dosing – 2 hours	4	4	17
1 hr. post-dose	2	4	14
2 hr. post-dose	1	5	13
9 hr. post-dose	0	5	10

*mean µmol/L whole blood; These values were taken from graphs provided in the paper.

Urine levels of isobutanol, isobutyraldehyde, and isobutyric acid found following isobutanol administration.

Sampling Time (hours)	Isobutanol*	Isobutyraldehyde*	Isobutyric Acid*
Beginning of dosing	0	0	0
End of dosing – 2 hours	30	4	80
1 hr. post-dose	125	6	70
2 hr. post-dose	100	6	70
8 hr. post-dose	50	7	40
9 hr. post-dose	10	6	30

*mean µmol/L urine; These values were taken from graphs provided in the paper.

Reliability: (score = 2)
Reference: Rudell, E. von, Bonte, W., Sprung, R., and Kuhnholz, B. (1983) "Zur Pharmakokinetik der holheren aliphatischen Alkohole." Beitr. Gerichtl. Med., Vol. 41, 211-218.

(b) Preferred value
Species: human (6 subjects)
Strain: N/A
Sex: Two males and four females
Route of Admin: oral
Exposure Period 30 minutes
Frequency of Treatment: Single
Duration of Test : Four hours
Exposure Concentration 1875 mg/L Isobutanol & 30% (by vol.) Ethanol in distilled water
Control Group: None (biological samples taken prior to exposure)
Method: In an effort to understand the elimination kinetics of aliphatic alcohols found in alcoholic beverages, research was conducted with human subjects. Test subjects consumed a beverage containing 1875 mg/L isobutanol and 30% ethanol over a 30 minute time period. This exposure resulted in an oral dose of approximately 5 mg/kg isobutanol and 0.80 g/kg ethanol. Blood samples were collected prior to consumption, at 30, 45, 60, 90, 120, 145, 180, 210, and 240 minutes after the start of the exposure. The blood samples were analysed by gas chromatography. Blood concentration-time curves were constructed for isobutanol and ethanol.

Year: 1990
GLP: no
Test substances: isobutanol, ethanol, propanol, methanol
Purity: not provided
Result: The blood concentrations of isobutanol peaked at 45 minutes after the start of the exposure period. The addition of ethanol to the test beverage altered the rate of isobutanol metabolism (via a competition for metabolic enzymes). Blood levels of isobutanol decreased over the remaining time periods. The T1/2 for isobutanol (in the presence of large amounts of ethanol) was 1.46 hours. Peak serum levels of isobutanol were approximately "6 mg/kg" while the blood ethanol levels were reported as approximately "1%"
Reliability: Score = 2, valid with restrictions
Reference: Bilzer, N., Schmutte, P., Jens, M., and Penners, B-M. (1990) "Kinetik aliphatischer Alkohole (Methanol, Propanol-1, und Isobutanol) bei Anwesenheit von Athanol im menschlichen Korper". (The kinetics of aliphatic alcohols (methanol, propanol-1, and isobutanol) in presence of ethanol in human body"). Blutalkohol, Vol. 27, No. 6, pp.385-409.

(c) Species: Human
Strain: N/A
Sex: unknown
Route of Admin: N/A (in vitro)
Exposure Period 10 minutes
Freq. of Treatment: Single
Duration of Test: 10 minutes
Exposure Conc.: 100 μM
Control Group: compared to 2.5 to 10 mM ethanol

	Method:	The roles of different isozymes of alcohol dehydrogenase (ADH) in the metabolism of aliphatic alcohols were investigated. Human liver ADH isoenzymes were prepared from two healthy tissue donors that succumbed to sudden death. Class I, II, and III ADH isoenzymes were isolated using DEAE-cellulose chromatography with affinity chromatography as the final separation step. The enzymes were assayed at 25°C in 50 mM sodium phosphate buffer at pH 7.4 containing 1.5 mM NAD and the respective alcohols. 50 mM semicarbazide was used to prevent the further reaction of the aldehydes to the corresponding acids. The reaction was initiated by the addition of the isoenzyme and stopped by the addition of ortho-phosphoric acid. The addition of the acid also liberated the respective aldehydes that were then analysed in the vial headspace by gas chromatography. All runs were assayed in triplicate. The reaction time was such that the aldehyde increased linearly with isoenzyme concentration. An additional check was to correlate the concentration of the aldehyde with the increase in NADH concentration (determined spectrophotometrically). Kinetic constants were estimated from the initial rate equations using a simplex algorithm with standard deviations estimated using Monte Carlo sensitivity analysis.
	Year:	1988
	GLP:	no
	Test substance:	isobutanol
	Result:	Class I ADH had a Km of 33 µM and a Vmax of 0.19 IU/mg protein for isobutanol. The resulting Class I activity (IU/mg) was 0.14 while the Class II ADH activity was 0.0004. Class III activity was below the limit of detection. These results demonstrate that the Class I ADH activity is primarily responsible for the oxidation of isobutanol in the human liver and that isobutyraldehyde is the product of the reaction.
	Reliability:	(score = 2)
	Reference:	Ehrig, T., Bohren, K.M., Wermuth, B., and von Wartburg, J-P. (1988) "Degradation of Aliphatic Ethanol and Pharmacokinetic Implications." Alcoholism: Clinical and Experimental Research, Vol. 26, No. 6, pp. 789-794.
(d)	Species:	Human, rat, chick embryo
	Strain:	Human - N/A, rat – Sprague-Dawley, chick embryo - unknown
	Sex:	Human and chick embryo – unknown, rat - female
	Route of Admin:	N/A (in vitro)
	Exposure Period	40 seconds
	Freq. of Treatment:	Single
	Duration of Test	40 seconds
	Exposure Concentration	100 µM
	Control Group:	0.8 to 3 mM ethanol
	Method:	The relative metabolic rate constants of aliphatic alcohol metabolism by liver supernatants from several species were investigated. Supernatants (100,000 g) prepared from human hepatocytes from four tissue donors were measured for alcohol dehydrogenase (ADH) activity. The supernatants were prepared in Hepes-DTT-sucrose buffer. The supernatants were diluted in a Tris-phosphate buffer (ph = 7.3) assayed at 38°C after the addition of 3 mM NAD+ and isobutanol. The rates of NADH formation were followed at 340 nm for 40 seconds at each substrate concentration using a spectrophotometer. Semicarbazide was used to prevent the further reaction of the aldehydes to the corresponding acids. All reactions followed the Michealis-Menten kinetics and Vmax and Km was calculated using the Lineweaver-Burke method.
	Year:	1990
	GLP:	no

	Test substance:	isobutanol
	Result:	Rat liver supernatant ADH activity had a Km of 0.05 μM and a Vmax of 1.07 μmol min^{-1} g wet $wt.^{-1}$. Human liver supernatant ADH activity had a Km of 0.04 – 0.11 μM and a Vmax of 0.68 – 0.86 μmol min^{-1} g wet $wt.^{-1}$. Chick embryo liver supernatant ADH activity had a Km of 0.22 μM and a Vmax of 0.29 μmol min^{-1} g wet $wt.^{-1}$.
	Reliability:	(score = 2)
	Reference:	Sinclair, J., Lambrecht, L., and E.L. Smith (1990) "Hepatic Alcohol Dehydrogenase Activity in Chick Hepatocytes Towards the Major Alcohols Present in Commercial Alcoholic Beverages: Comparison with Activities in Rat and Human Liver." Comp. Biochem. Physiol. Vol. 96B, No. 4, pp.677-682.
(e)	Species:	Rat
	Strain:	Wistar
	Sex:	Male and Female
	Route of Admin:	Intraperitoneal, liver perfusion, in vitro liver homogenate
	Exposure Period	In vivo – 7 hours, perfusion – 60 minutes, in vitro – 30 minutes
	Freq. of Treatment:	Single
	Duration of Test	Up to 7 hours
	Exposure Concentration	In vivo – 237 mg/kg isobutanol, 1,569 mg/kg ethanol Perfusion – 26.5 mmoles/liter isobutanol and ethanol, in vitro – 11 mM isobutanol, 1100 mM ethanol
	Control Group:	In vivo – pre-injection samples, perfusion and in vitro - yes
	Method:	The metabolism of isobutanol in rats was investigated. In vivo – two rats received an intraperitoneal injection of isobutanol and ethanol. Blood samples were collected via the tail vein after 15, 45, 75, and 105 minutes and then after every hour for up to 7 hours and analysed for each of the alcohols by gas chromatography. Perfusion – rats were anaesthetized with Nembutal (demonstrated not to interfere with ethanol metabolism) and the hepatic portal vein and the hepatic vein were cannulated. A blood:saline mixture to which isobutanol and ethanol had been added (final concentration of each - 26.5 mmoles/liter) was used to perfuse (2 ml/minute) the liver in situ for 60 minutes. These experiments were repeated with 2mM pyrazole added to the mixture. Samples were collected at 15-minute intervals and analysed by gas chromatography for each of the alcohols. In vitro – A supernatant was produced by homogenizing adult rat livers followed by centrifugation at 800 x *g* for five minutes. Isobutanol was added (either alone or with ethanol) with 2 mM NAD to initiate the reaction. These experiments were repeated with 2mM pyrazole added to the mixture. The incubation flasks were shaken at 30C for 30 minutes with samples taken for analysis for gas chromatography at 0, 15, and 30 minutes. Pyrazole was added to both the in situ and in vitro experiments to inhibit the metabolism of the alcohols by alcohol dehydrogenase.
	Year:	1969
	GLP:	no
	Test substance:	Isobutanol and ethanol
	Result:	Isobutanol reached levels in blood of approximately 0.1 mg/ml at 15 minutes post-injection and these levels decreased only slightly over the 7 hour test period. Blood ethanol levels reached peak levels of 0.7 mg/ml and decreased over 5 hours to baseline. Blood levels of isobutanol did not start to decline appreciably until ethanol blood levels reached 0.2 mg/ml. Pyrazole inhibited the metabolism of both isobutanol and ethanol by the same degree, supporting the idea that both of these alcohols were metabolized by alcohol dehydrogenase. Rat liver perfusion experiments

results indicated that isobutanol was metabolized at a rate of 0.06 mM isobutanol/gram of liver during the first 30 minutes. Isobutanol was metabolized more rapidly than ethanol or isoamyl alcohol but slower than n-propanol in the perfused rat liver. Liver homogenates from male and female rats metabolized both ethanol and isobutanol at equal rates, demonstrating a lack of gender differences for metabolism. Pyrazole inhibited the metabolism of both ethanol and isobutanol by alcohol dehydrogenase in vitro. Isobutanol was metabolized by the rat liver homogenate in vitro system at a rate of 0.2 mM/g liver in 30 minutes. Similar to the liver perfusion results, isobutanol was metabolized more rapidly than ethanol or isoamyl alcohol but slower than n-propanol in the in vitro system.

Reliability: (score = 2)

Reference: Hedlund, S-G. and Kiessling, K-H. (1969) "The Physiological Mechanism Involved in Hangover 1. The Oxidation of Some Lower Aliphatic Fusel Alcohols and Aldehydes in Rat Liver and Their Effects on the Mitochondrial Oxidation of Various Substrates" Acta Pharmacol. Et Toxicol. Vol.27, pp. 381-396.

(f) Species: Rabbit

Strain: Not available

Sex: Male

Route of Admin: Oral

Exposure Period Single administration

Freq. of Treatment: Single

Duration of Test Six hours

Exposure Concentration 2 ml/kg

Control Group: None

Method: The metabolism of isobutanol in rabbits was investigated. Anaesthetized male rabbits were administered isobutanol and arterial blood samples were taken at 30 minutes, and 1, 2, 3, 4, 5, and 6 hours post-dosing. Blood levels of isobutanol were analysed by gas chromatography. A separate group of animals were evaluated for excretion of isobutanol and metabolites in the urine and exhaled air. Urine was collected via a bladder catheter while exhaled air was collected with a mask and one-way valve. Levels in urine and expired air were measured by gas chromatography. Rabbit liver microsomes were prepared and the ability of this in vitro preparation to metabolise isobutanol was determined. An additional experiment described rabbits dosed orally with 2 ml/kg ~~of~~ isobutanol followed by consumption of water containing 20% (v/v) isobutanol. Urine was collected and analysed by gas chromatography.

Year: 1975

GLP: no

Test substance: isobutanol

Result: Isobutanol blood levels peaked at 1 hour post-dosing with blood levels of approximately 0.8 mg/ml. Blood levels decreased over the next 3 hours and were near zero by 4 hours post-dosing. Blood pH levels dropped to 7.2-7.3 from the 30-minute time point until 4 hours post-dosing. Changes in blood pH were considered due to depressed respiratory activity and not due to the production of metabolites (e.g. isobutyric acid). Rabbit liver homogenates metabolized isobutanol at rates approximately equal to ethanol (results of a previous experiment). Very little (0.5%) of the isobutanol administered orally was excreted in the urine or exhaled air. Urinary levels of isobutyraldehyde were 0.12 mg/ml while isobutyric acid was present in trace amounts. Unexplainable levels of isovaleric acid (1.6 mg/ml) were

		found in the urine of the rabbits receiving oral dose of isobutanol and isobutanol in the drinking water. The metabolite described as isovaleric acid may have been another metabolite (described in the paper by Rudell, et al.; see (a)) co-eluting with isovaleric acid on the chromatogram.
	Reliability:	(score = 2)
	Reference:	Saito, M. (1975) "Studies On The Metabolism Of Lower Alcohols" N.U. Med. J. Vol. 34, pp. 569-585.
(g)	Species:	Human
	Strain:	Not available
	Sex:	Not available
	Route of Admin:	in vitro
	Exposure Period	Single administration
	Freq. of Treatment:	Single
	Duration of Test	Not available
	Exposure Concentration	1 nM
	Control Group:	Compared to ethanol
	Method:	The metabolism of isobutanol in human skin samples was investigated. Homogenates of human skin were prepared and alcohol dehydrogenase activities determined for a series of alcohols. Attempts were made to correlate enzyme activity with the frequency of erythemogenesis observed in a test population of human subjects.
	Year:	1987
	GLP:	no
	Test substance:	isobutanol
	Result:	Human skin alcohol dehydrogenase enzymatic activity for isobutanol was 103.7 nM/mg protein-minute. Corresponding values for ethanol were 98.1 nM/mg protein-minute. Two of twelve test subjects had erythemogenic reactions to isobutanol.
	Reliability:	score=2
	Reference:	Wilkin, J.K. and Stewart, J.H. (1987) "Substrate Specificity of Human Cutaneous Alcohol Dehydrogenase and Erythema Provoked by Lower Aliphatic Alcohols" J. Invest. Dermatol. Vol. 88, pp. 452-454.
(h)	Preferred value	
	Species:	rat
	Strain:	Sprague-Dawley
	Sex:	male
	Route of Admin:	inhalation
	Exposure Period	Two hours
	Freq. of Treatment:	Single
	Duration of Test	two hours
	Exposure Concentration	2000 ppm (the chamber is charged with 2000 ppm isobutanol and the concentration drops as the rat inhales the test article. Loss to chamber equipment and external surface of the rat is corrected for).
	Control Group:	None (biological samples taken prior to exposure). The amount inhaled by the rat (versus deposited on chamber equipment surfaces is corrected for).
	Method:	In an effort to understand the respiratory bioavailability of aliphatic alcohols and esters, a whole-body plethysmograph was installed in a gas-uptake chamber. The rat has an indwelling jugular cannula implanted prior to study start and is placed in the plethysmograph. The plethysmograph (containing the rat) is then placed in the gas-uptake chamber. The leads from the plethysmograph and the venous catheter are exteriorized from the chamber for sample and data collection. The chamber is charged with 2000-ppm

isobutanol and the chamber concentration decay curve is followed by gas chromatography. In addition, venous blood samples are taken at 0, 5, 10, 20, 25, 30, 40, 50, 60, and 90 minutes. The whole-body plethysmograph is designed to measure (non-invasively) ventilatory movements on conscious rats. By collecting data on ventilatory movements, and chamber and venous blood isobutanol concentrations, respiratory bioavailability determinations can be calculated. Blood samples were analyzed for isobutanol (N=7) and isobutyric acid (N=2) concentrations.

Year: 2003
GLP: no (conducted in spirit of GLP, but not specifically)
Test substances: isobutanol
Purity: Spectroscopic grade (>99.9%)
Result: The blood concentrations of isobutanol and isobutyric acid during the exposure period are reported below. The presence of isobutyric acid following isobutanol inhalation exposure clearly demonstrates that isobutyric acid was the major metabolite of isobutanol metabolism. Blood levels of isobutanol increased up to 277 µM at 15 minutes into the exposure, and declined over the remaining 70 minutes. Chamber concentrations decline from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Isobutyric acid levels increased up to 93 µM at 25 minutes, after which they declined to 40 µM at 60 minutes.

Isobutanol and isobutyric acid blood levels found following isobutanol inhalation.

Sampling Time (minutes)	Isobutanol*	Isobutyric Acid*
0	0	0
5	169	8
10	254	18
15	278	43
20	264	55
25	240	93
30	252	91
40	248	42
50	233	39
60	243	40
90	155	ND

*mean µM whole blood (N=7 for isobutanol; N= 2 for isobutyric acid)

Reliability: Score=2, valid with restrictions
Reference: Poet, T. (2003) Unpublished data. Battelle, Pacific Northwest National Laboratory, US Dept. of Energy. For Oxo-Process Panel, Chemstar, American Chemistry Council, Arlington, VA, 22209.